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Temporal summation and a C-fibre reflex in the rat: effects of morphine on facilitatory and inhibitory mechanisms

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Abstract

In intact rats, an inhibitory mechanism counteracts the increase in excitability of a flexor reflex, which is seen in spinal animals following temporal summation of C-fibre inputs; the Rostral Ventromedial Medulla is involved in this inhibitory mechanism. Electromyographic responses elicited by electrical stimulation of the sural nerve were recorded from the biceps femoris in four types of preparations, namely intact, sham-operated, Rostral Ventromedial Medulla-lesioned and decerebrate-spinal rats. The excitability of the C-fibre reflex was tested during and following high intensity homotopic electrical conditioning stimuli. Morphine (2 mg/kg) did not significantly change the basal test response but increased the excitability of the spinal cord during conditioning. This effect was triggered by the strength of inputs, involved the Rostral Ventromedial Medulla and was probably related to some forms of motor stimulation through dopaminergic transmission. While wind-up was not reduced, the inhibition related to Diffuse Noxious Inhibitory Controls, which occurred following the conditioning period, did. In spinal animals where inhibitory mechanisms disappear, the depressive effects of morphine were unmasked for both wind-up and post-conditioning facilitations. All effects of morphine were completely reversed by naloxone.

Keywords: C-fibre; Flexor reflex; Rostral ventromedial medulla; Wind-up; Spino-bulbo-spinal; Inhibitory feedback mechanism; Morphine

1. Introduction

Sensitisation of spinal cord neurones by repeated or sustained nociceptive inputs, has been studied extensively in recent years (see Refs. Dougherty et al., 1993; Dubner and Basbaum, 1994). For convenience, electrical stimuli have often been used to generate such facilitations and the neuronal or reflex responses have been studied during or following conditioning peripheral stimuli. In this context, Mendell and Wall (1965) described the phenomenon of ''wind-up'', which consists of a progressive increase in neuronal or reflex responses to a constant level of nociceptive stimulation applied repetitively to the same area of the body. A related phenomenon was observed by Woolf and Wall (1986) in decerebrate spinal animals: following 20 s of 1-Hz electrical stimulation of C-fibres homotopically, a marked facilitation of a flexion reflex occurred. This observation suggested that temporal summation of brief C-fibre afferent inputs within the spinal cord, can elicit a prolonged hyperexcitability of neurones involved in the transmission of nociceptive signals (see also references in Gozariu et al., 1997).

In a previous study (Gozariu et al., 1997), we showed that the effects of temporal summation of C-fibre inputs on a C-fibre reflex, were very dependent upon the type of preparation. In intact rats, the C-fibre reflex elicited by stimulation of the sural nerve was facilitated during the conditioning period (20 s of homotopic electrical stimulation of the sural nerve) but was then inhibited in a stimulus-dependent manner for a long period of time. In spinal rats, such inhibitions were replaced by long-lasting facilitations, as expected. Our conclusion was that in intact animals, we were dealing with both facilitatory and inhibitory mechanisms, the former being organised at the spinal level and the latter involving supraspinal mechanisms. In a further study (Gozariu et al., 1998), we observed that the inhibitory processes in intact animals were greatly reduced by lesions of the rostral Rostral Ventromedial Medulla, a major source of descending inhibitory

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controls (Fields and Basbaum, 1989, 1994). However, additional mechanisms were probably involved because large lesions of the Rostral Ventromedial Medulla did not completely block the inhibition.

Diffuse Noxious Inhibitory Controls are another source of spino-bulbo-spinal inhibition (Le Bars and Villanueva, 1988). Since Diffuse Noxious Inhibitory Controls are very sensitive to morphine (Le Bars et al., 1995), we tested the effects of this drug on the model described above in intact animals. Both the effects observed during and following the conditioning procedure of temporal summation were analysed. In order to determine the nature of the residual post-conditioning inhibition seen following Rostral Ventromedial Medulla-lesions, we compared the effects of morphine in sham-operated and Rostral Ventromedial Medulla-lesioned rats. Finally, we studied a group of spinal animals to allow a comparison with previous reports (Woolf and Wall, 1986).

Since our goal was to study the effects of morphine on phenomena elicited by temporal summation, it was essential to use a dose, which did not affect the basal activity of the test C-fibre reflex triggered by a stimulus intensity of 1.5 × the threshold (1.5 T). We chose a dose of 2 mg/kg, which exerted no significant effect on the reflex at low intensities of stimulation. Indeed, intravenous morphine exerts different effects on the C-fibre reflex in a dose-dependent manner (Guirimand et al., 1995), low (0.5–1 mg/kg) and high doses (4–10 mg/kg) inducing facilitation and depression, respectively. Since these effects of morphine are also determined by the stimulus strength, a compromise had to be found.

2. Materials and methods

2.1. General procedure

Experiments were performed on four groups of Sprague–Dawley rats weighing 300–400 g, namely: (1) intact; (2) Rostral Ventromedial Medulla-lesioned, (3) sham-operated; and (4) those which had been decerebrated at the mid-collicular level and spinalised. Experiments were carried out according to the ethical recommendations of the Committee for Research and Ethical Issues of the International Association for the Study of Pain.

In the Rostral Ventromedial Medulla and sham-operated groups (10 rats), electrolytic lesions were made in the brain; electrophysiological experiments were performed 1 week later; and finally the brain lesions were reconstructed after the examination of serial histological sections. In order to make the electrolytic lesions, the animals, under chloral hydrate anaesthesia (450 mg/kg; i.p.), were placed in a stereotaxic frame. The lesions of the Rostral Ventromedial Medulla were made by passing cathodal current (5 mA) for 8 s through a stainless steel electrode insulated except for 0.5 mm at its tip. The tip was located at the following co-ordinates as defined by Paxinos and Watson (1986): 11 mm caudal to bregma, 0 mm lateral to the midline and 0.2 mm below the interaural axis. In the sham-operated animals, the electrode was lowered but current was not passed. We used electrolytic, complete lesions of the Rostral Ventromedial Medulla because of the very high fatality level, which occurs following injections into the medulla of neurotoxic drugs such as ibotenic or quinolinic acid. Attempts to destroy the Rostral Ventromedial Medulla throughout its whole rostro-caudal extent with neurotoxic drugs always resulted in the death of the animal (Bouhassira et al., 1993a). It follows that we cannot exclude the possibility that our results were, at least partly, due to the lesioning of passing fibres.

2.2. Preparation for electrophysiology

Before electrophysiological recordings were made, all the rats were deeply anaesthetised with 2% halothane in a nitrous oxide/oxygen mixture (2/3:1/3). A tracheotomy was performed and the animals were artificially ventilated through a tracheal cannula.

One group of six rats was decerebrated at the mid-collicular level by suction of the brain contents rostral to the mid-collicular region. These animals were then spinalised at the T_8–T_{10} level after exposure of the cord by laminectomy.

Following surgery, the anaesthesia was abolished in the decerebrate spinal rats and the concentration of halothane was lowered to 0.9% in 100% oxygen in the other groups. Throughout the experiments, the heart rate and the parameters of ventilation and anaesthesia were monitored continuously. The respiratory rate (50 counts/min) and levels of O_2, end-tidal CO_2, (3.2–3.5%) and halothane (0.9%) were monitored using a capnometer (Capnomac II, Datex Instruments, Helsinki, Finland) and each was under the control of an alarm. Body temperature was maintained at 37.5 ± 0.5°C by means of a homeothermic blanket system.

2.3. Electrophysiological recordings

Electrophysiological recordings were made as described previously (Gozariu et al., 1997) from the ipsilateral biceps femoris muscle, of a C-fibre evoked reflex elicited by electrical stimulation within the receptive field of the sural nerve. The test stimuli were single square-wave electrical shocks of 2-ms duration and constant level of stimulation, namely 50% above the threshold (1.5 T) of the C-fibre evoked response. These were delivered once every 6 s (0.17 Hz) from a constant-current stimulator. The conditioning stimuli (trains of 20 2-ms pulses) were delivered to the same area, at a constant intensity (10 × the threshold for the C-fibre reflex) and frequency (1 Hz). The stimulus intensities and electromyographic responses were fed to an oscilloscope for continuous monitoring and to a computerised system (PLS, Notocord) for on-line digitisation and processing.

2.4. Experimental procedure

After a control period of 10 min and following the conditioning period, the reflexes evoked by the test stimuli were recorded continuously for 20 min.

In each animal, 2 mg/kg morphine hydrochloride was then injected intravenously. This dose was chosen because it had no significant effect on the reflex response at low stimulus intensities (1–4 × T for C-fibres). Following a period of 15 min after the morphine injection during which the stability of the reflex response was checked, 20 conditioning stimuli were delivered. These conditioning stimuli had exactly the same characteristics as described before. Following the conditioning period, the reflex elicited by the test stimulus was again recorded for 20 min. Finally, 0.04 mg of naloxone hydrochloride was administered intravenously in all the animals and 5 min later, a further 20 conditioning stimuli were delivered. These conditioning stimuli were similar to those previously described. Following the conditioning period, the reflexes evoked by the test stimuli were again recorded for 20 min.

2.5. Histological controls

At the conclusion of the experiments, the sham-operated rats and those with Rostral Ventromedial Medulla-lesions were deeply anaesthetised with 3% halothane, and their brains perfused through the heart with 0.9% NaCl followed by 10% formaldehyde. The brain was then frozen, cut in serial 100 μm-thick sections, and Nissl stained with Cresyl violet or carmin. Brainstem lesions were reconstructed from camera lucida drawings of serial sections.

The total extent of the lesions in the Rostral Ventromedial Medulla is represented schematically in Fig. 1 for all the animals treated in this way. In each case, the lesions included the entire rostro-caudal extent of nucleus raphe magnus and most parts of the adjacent reticular nuclei,

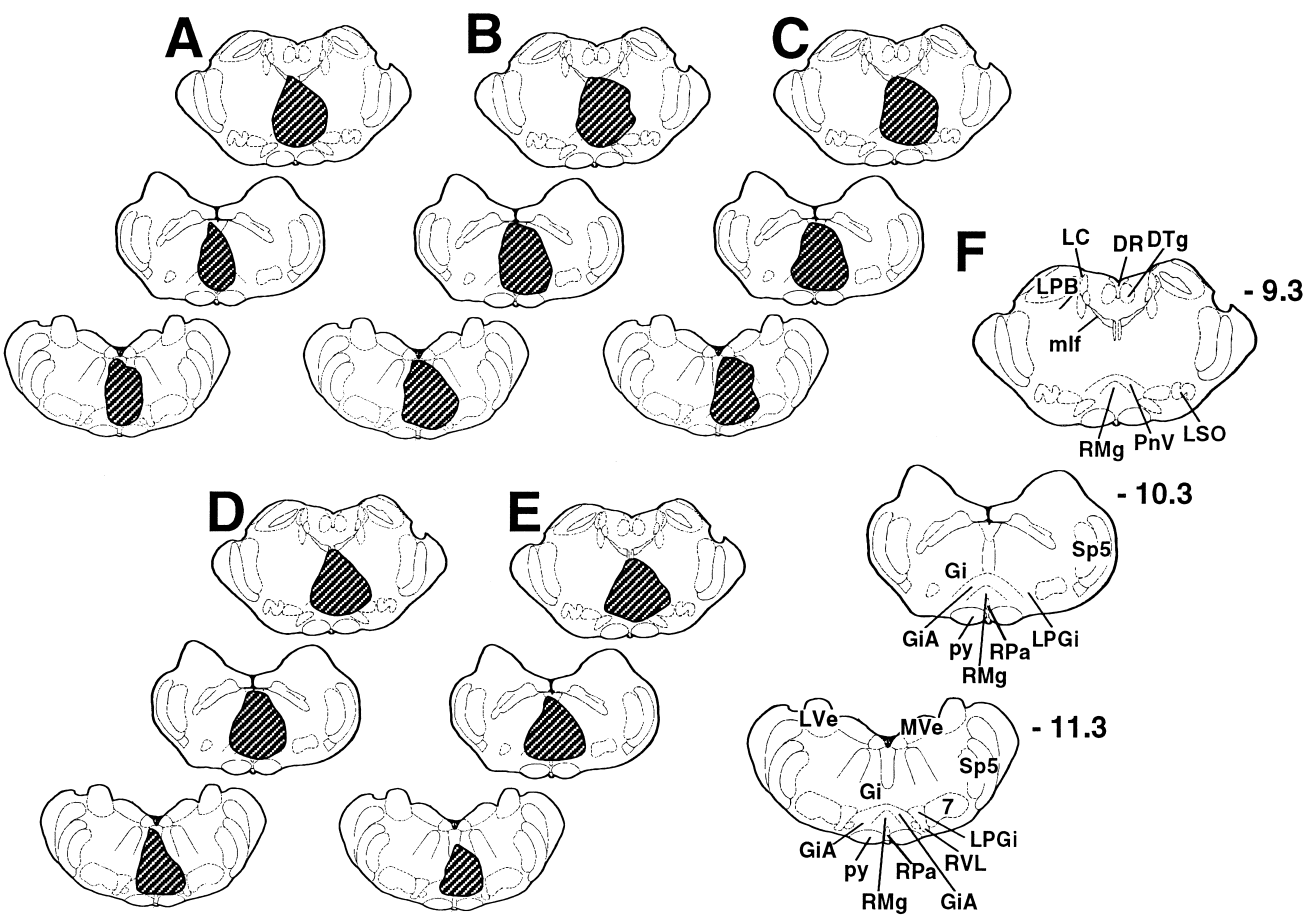

Fig. 1. Schematic representation of the lesions (hatched areas) of the Rostral Ventromedial Medulla in all the animals tested 1 week after lesioning (A–E). The drawings are simplified from Paxinos and Watson (1986). Keys for medullary areas are indicated by abbreviations in F: 7, facial nucleus; DR, dorsal raphe nucleus; DTg, dorsal tegmental nucleus; Gi, nucleus reticularis gigantocellularis; GiA, nucleus reticularis gigantocellularis pars alpha; LC, locus coeruleus; LPB, lateral parabrachial nucleus; LPGi, nucleus reticularis paragigantocellularis lateralis; LSO, lateral superior olive; LVe, lateral vestibular nucleus; mlf, medial longitudinal fasciculus; MVe, medial vestibular nucleus; py, pyramidal tract; PnV, ventral pontine reticular nucleus; RMg, nucleus raphe magnus; RPa, nucleus raphe palidus; RVL, rostroventral lateral reticular nucleus; sp5, spinal trigeminal nucleus. The levels indicated are caudal to bregma.

nucleus reticularis gigantocellularis pars alpha and nucleus paragigantocellularis.

2.6. Analysis of the conditioning stimulation paradigm

Each individual electromyographic response was expressed as a percentage of the mean control value, which was derived from the 20 successive C-fibre reflex responses in the 2-min period preceding the conditioning procedure. During the post-conditioning period, results were expressed as means of 10 successive individual responses obtained over a 1-min period. A two-way analysis of variance (ANOVA) for repeated measures followed by post-hoc Fisher posteriori least-significant difference (PLSD) tests were used for each preparation to compare the control time-courses with those following morphine and naloxone during both the conditioning and the post-conditioning periods. This was followed in each group by ANOVA and post-hoc Fisher (PLSD) tests. Two comparisons were made for the conditioning procedure: each conditioned response was compared with (1) both the last test-response and (2) the first response of the conditioning period; during the post-conditioning periods, the responses were compared with mean pre-conditioning control values. Data were expressed as means $\pm$ S.E.M. Results were considered significant when $P < 0.05$.

2.7. Drugs

The following drugs were used: Morphine Hydrochloride and Naloxone hydrochloride (Sigma). Doses are expressed in milligram per kilogram. The drugs were dissolved in saline to obtain adequate concentrations.

3. Results

3.1. Electromyographic signals and preparations

In the intact, the sham-operated and the Rostral Ventromedial Medulla-lesioned anaesthetised rats, electrical stimulation (2 ms, 0.17 Hz) within the sural nerve region elicited a two-component reflex response in the ipsilateral biceps femoris muscle. We quantitatively analysed the second component of this reflex within a time-window 100–450 ms after the stimulus onset. This component results from activation of unmyelinated cutaneous afferent C-fibres. The mean thresholds and the latencies and durations at 1.5 T for C-fibres were comparable in the intact, sham-operated and Rostral Ventromedial Medulla-lesioned rats. The thresholds were 5.8 ± 0.7, 5.2 ± 0.6 and 5.7 ± 0.8 mA, respectively; the latencies at 1.5 T were: 178.3 ± 9.1, 170 ± 14.2 and 155 ± 12.8 ms, respectively; while the durations of this C-fibre evoked reflex were: 203.3 ± 12.8, 234 ± 20 and 224 ± 25 ms, respectively.

In spinal (T_8–T_{10}) animals anaesthetised with 0.9% halothane, no clear electromyographic responses could be recorded. It was for this reason that these experiments were performed in non-anaesthetised animals, in which recordings started at least 1 h following the surgical procedure including the spinal section. The electromyographic signal was weaker than in the intact rats. A significantly lower threshold (mean: 1.6 ± 0.5 mA), a significantly shorter latency (mean: 111.5 ± 12.8 ms) and a longer, albeit not significantly, mean duration (251.7 ± 10.5 ms) were found by comparison with the other groups of (anaesthetised) animals.

In all the preparations, the C-fibre reflex elicited by electrical stimuli at 1.5 T was the test response. Neither morphine (2 mg/kg; i.v.) nor naloxone (0.04 mg/kg; i.v.) exerted a significant effect on the C-fibre control reflex at this constant intensity of stimulation.

The temporal summation of C-fibre inputs was studied during and after the conditioning procedure, which consisted of 20 pulses applied at a constant intensity (10 T) and frequency (1 Hz) through the same electrodes as the test stimuli. This was done in each group of animals, during the control period, following morphine and following naloxone.

We will successively describe the effects of morphine on the evolution of the C-fibre reflex during and following the conditioning period in non-transected animals. We will then describe the results obtained in spinal animals. These latter experiments were designed with reference to previously published studies (Woolf and Wall, 1986).

3.2. Effects of morphine on the C-fibre reflex during conditioning in non-transected animals

As shown in Fig. 2A for the intact rats, during control conditioning, the C-fibre reflex progressively increased with the first five conditioned stimuli (wind-up phenomenon), reached a plateau between the 6th and 10th stimuli and then decreased gradually from one stimulus to the next to reach a value at the end of conditioning, which was close to that of the first conditioned response. Following the administration of morphine, there was an overall shift of the curve to above that for the corresponding control. The reflex responses reached a maximum following the 4th stimulus and then decreased progressively from the 5th until the 20th stimulus. Naloxone completely reversed the effects of morphine. The effects observed in the sham-operated animals are shown in Fig. 2B and were essentially similar to those found in the intact rats.

In the Rostral Ventromedial Medulla-lesioned rats (Fig. 2C), the results were completely different both in terms of the conditioned responses and of the pharmacological effects. During control conditioning, a wind-up phenomenon was seen for the first five conditioned stimuli, which then plateaued for the rest of the conditioning period. Although there was no significant difference between the sham-oper-

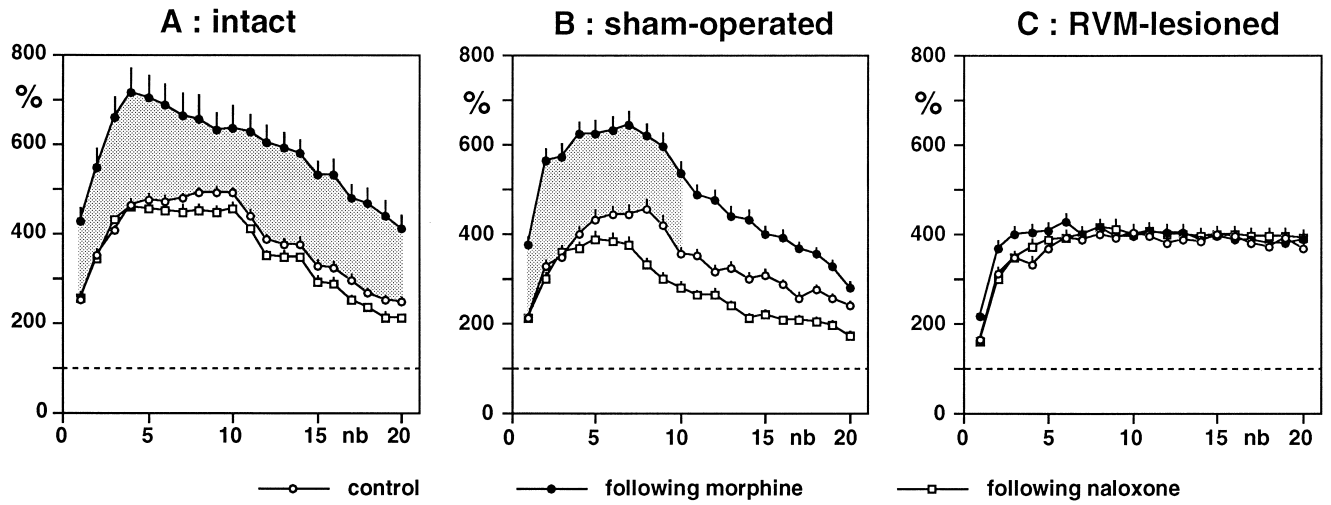

Fig. 2. Mean curves showing the effects of intravenous morphine (2 mg/kg), followed by naloxone (0.04 mg/kg) on the temporal evolution of the C-fibre reflex during the conditioning procedure in three different preparations, namely intact (A), sham-operated (B) and Rostral Ventromedial Medulla-lesioned (C) animals. The conditioning stimuli applied to the sural nerve region were 20 2 ms-duration pulses delivered at 1 Hz and an intensity of 10× the threshold for the C-fibre reflex. Each individual C-fibre response (ordinate) was expressed as a percentage of the mean control value recorded during the 2-min period preceding the conditioning procedure. The abscissa shows the rank order (number: nb) of the conditioning stimulus. Shaded areas indicate significant differences between the control and the post-morphine curves. (A) In intact rats, the C-fibre reflex was facilitated from one stimulus to the next for the first few stimuli, then plateaued and finally decreased gradually. Following morphine, all the reflex responses were higher during conditioning with a time-course similar to the control curve. Naloxone completely reversed these effects. (B) In sham-operated rats, the results were roughly similar to that seen in the intact group with no statistical differences between homologous curves. (C) In Rostral Ventromedial Medulla-lesioned rats, the C-fibre reflex increased continuously from one stimulus to the next for the first few stimuli and then plateaued for the remainder of the conditioning period. Following morphine and naloxone, the corresponding time-courses were superimposed on the mean control curve.

ated and Rostral Ventromedial Medulla-lesioned rats for the first 11 conditioned responses, the remaining nine (i.e. the 12th–20th) were significantly higher in the Rostral Ventromedial Medulla-lesioned animals. Following morphine and subsequent naloxone, the curves describing the mean evolution of the reflex responses during condition-

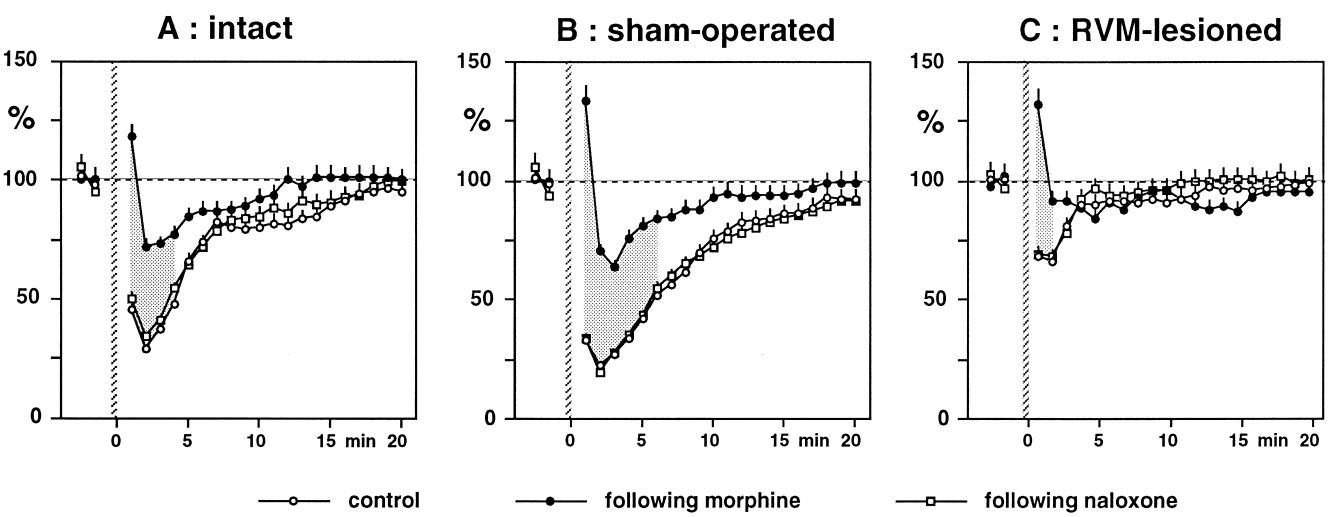

Fig. 3. Mean curves showing the effects of intravenous morphine (2 mg/kg), followed by naloxone (0.04 mg/kg) on the temporal evolution of the C-fibre reflex following the conditioning procedure in three different preparations, namely intact (A), sham-operated (B) and Rostral Ventromedial Medulla-lesioned (C) animals. These are the same experiments as in Fig. 2, but analysed in the post-conditioning periods. In each individual case, the C-fibre reflex was calculated as a percentage of the mean control value recorded during the 2-min period preceding the conditioning procedure and the results were then expressed as means of 10 successive individual responses, which corresponded to a 1-min period. Abscissa: time (min) following the conditioning procedure. Shaded areas indicate significant differences between the control and the post-morphine curves. Hatched areas indicate the 20-s period of conditioning. (A) The strong post-conditioning inhibition observed in intact rats was significantly reduced by morphine in a naloxone-reversible fashion. (B) Similar results were observed in sham-operated rats. (C) In Rostral Ventromedial Medulla-lesioned rats, the control post-conditioning inhibition was reduced to a great extent. The remaining, slight, post-conditioning inhibition was completely blocked by morphine and restored by naloxone.

ing, were more or less superimposed on the mean control curve.

3.3. Effects of morphine in the post-conditioning period in non-transected animals

As shown in Fig. 3A for the intact rats, when the test stimuli were again applied during the post-conditioning period, the C-fibre evoked reflex was strongly inhibited for a long period of time. This inhibition was significant during the period from 2 to 14 min following conditioning but there was a complete recovery by 20 min. Such strong post-conditioning effects were reduced by morphine: a significantly lower inhibition was seen and recovery was complete by 15 min. Naloxone completely reversed the effects of morphine. The effects observed in the sham-operated animals are shown in Fig. 3B and were essentially similar to those described in the intact rats.

In the Rostral Ventromedial Medulla-lesioned rats (Fig. 3C), the results were again completely different both in terms of the conditioned responses and of the pharmacological effects. Following the control conditioning, a slight but significant inhibition was seen for 2–3 min after conditioning; there was then a complete recovery within 10 min. These responses were significantly different from the sham-operated group during the first 7 min of the post-conditioning period. Following morphine administration, the residual inhibitory effect was completely blocked. This blockade was totally reversed following naloxone.

3.4. Effects of morphine on the C-fibre reflex during and following conditioning in spinal animals

In the spinal animals, the results were very different from those described above for the non-transected animals in terms of both the conditioned responses — either during or following conditioning — and the pharmacological effects. The evolution of the C-fibre reflex during and following conditioning are presented in Fig. 4A and B, respectively.

During the whole control conditioning period (Fig. 4A), the C-fibre reflex increased progressively from one stimulus to the next, with a maximum facilitation being observed following the final (20th) stimulus. Following morphine, the first five conditioned responses were not significantly different from those recorded during the control conditioning. However thereafter, the wind-up phenomenon was very much diminished. Naloxone completely reversed these effects.

During the post-conditioning period (Fig. 4B), a facilitation of the C-fibre reflex was seen. Following the control conditioning, a strong significant facilitation was observed, which progressively and completely recovered within 20 min. Following morphine, this post-conditioning facilitation was very much reduced and was significant only in the period from 1 to 6 min following conditioning. This effect of morphine was completely reversed by naloxone.

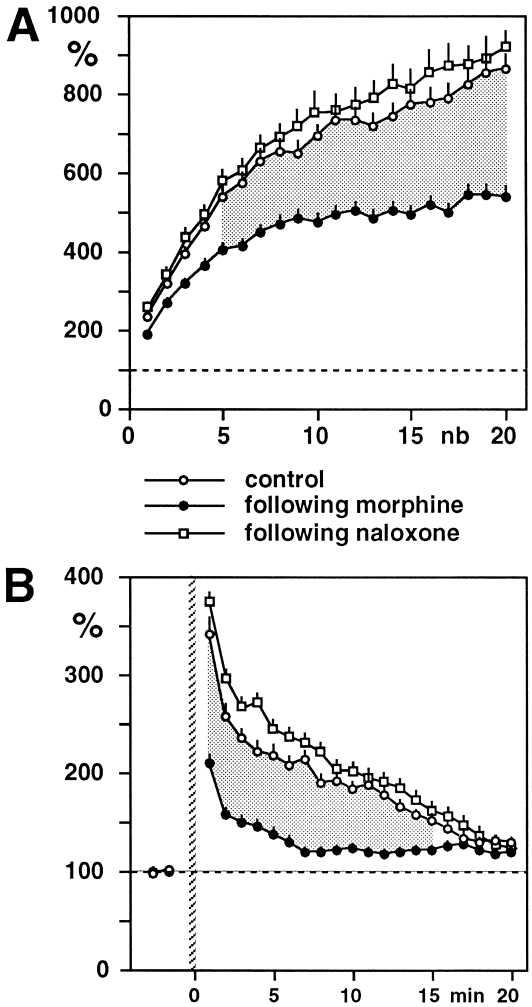

Fig. 4. Mean curves showing the effects of intravenous morphine (2 mg/kg), followed by naloxone (0.04 mg/kg) on the temporal evolution of the C-fibre reflex during (A) and following (B) the conditioning procedure in spinal animals. Experimental protocol and presentation as in Figs. 2 and 3. (A) During the conditioning procedure. During the control conditioning, the C-fibre reflex progressively increased from one stimulus to the next for the whole period. A similar, but significantly lower increase was seen following morphine. Naloxone completely reversed this effect of morphine. The curves were significantly different between the 5th and 20th stimuli. (B) Following the conditioning procedure. Following control conditioning, significant and strong facilitatory effects were seen for a long period of time. After morphine administration, this post-conditioning facilitation was very much reduced and naloxone completely reversed this effect of morphine. The curves were significantly different during the first 15-min post-conditioning.

4. Discussion

We have studied the effects of morphine on the temporal summation of C-fibre inputs on a C-fibre reflex using a dose that did not significantly change the basal test re-

sponse. The results we obtained were very dependent upon the type of preparation. In intact and sham-operated rats, morphine strongly facilitated the C-fibre reflex during the conditioning period and reduced the inhibition observed during the post-conditioning period. In Rostral Ventromedial Medulla-lesioned rats, the morphine-induced facilitations disappeared completely while the post-conditioning inhibition was reduced and further blocked by morphine. In spinal unanaesthetised animals, both the wind-up seen during conditioning and the post-conditioning facilitation were reduced by morphine. Naloxone completely reversed all effects of morphine.

We deliberately chose a 2-mg dose of morphine because it has been shown not to exert a significant effect on the C-fibre reflex at low intensities of stimulation (1–4 T) in intact rats (Guirimand et al., 1995). This was confirmed in the present study in the intact, sham-operated or Rostral Ventromedial Medulla-lesioned rats. This dose is in effect a ''turning point'' in that lower and higher doses facilitate and depress the C-fibre reflex, respectively (Guirimand et al., 1995).

Since the effects of Rostral Ventromedial Medulla lesions and spinal transection on the control responses were already discussed in previous papers (Gozariu et al., 1997, 1998), we will be focused here on the effects of morphine. The discussion will be organised into several sections as follows: the effects of morphine during conditioning in non-transected animals, during the post-conditioning period in non-transected animals and on the C-fibre reflex in spinal animals, conclusions and functional implications.

4.1. Effects of morphine during conditioning in non-transected animals

In both the intact and the sham-operated rats, morphine increased, in a naloxone-reversible fashion, the excitability of the motoneuronal pool during high intensity, high frequency stimulation. Interestingly, we observed a global shift of the appropriate curve towards upper values following morphine. Two conclusions can be drawn from these observations: (1) the morphine-induced increased excitability was not triggered by temporal summation but rather by the strength of the nociceptive inputs; (2) the decreasing phase was not sensitive to the opioid. This later observation strongly suggests that such a phenomenon was not related to Diffuse Noxious Inhibitory Controls that is very sensitive to this drug (see below). In addition, wind-up was not reduced by this dose of morphine.

In both rats and monkeys, systemic morphine produces dose-dependent biphasic effects on nociceptive flexion reflexes assessed with electromyographic methods, with facilitatory and depressive effects at lower and higher doses, respectively (Cooper and Vierck, 1986; Guirimand et al., 1995; Yeomans et al., 1995). In the present study, the intensity of stimulation during conditioning was $10\times$ threshold. The facilitatory effect of morphine was therefore consistent with our previous observations that the morphine-induced facilitations of the C-fibre reflex are intensity-dependent with no effects on the threshold and increasing effects at increasing intensities of stimulation (Guirimand et al., 1995).

The facilitatory effects of morphine disappeared completely in Rostral Ventromedial Medulla-lesioned rats. Two conclusions can be drawn from these observations: (1) the morphine-induced increased excitability seen in intact and sham-operated rats resulted from the activation of supraspinal opioid receptors; (2) such phenomena involved the Rostral Ventromedial Medulla, although we cannot exclude completely the possibility that our results were, at least partly, due to the lesioning of passing fibres.

Neuronal activities recorded in the Rostral Ventromedial Medulla are sensitive to the systemic administration of morphine. Thus, both spontaneous and noxious heat-evoked activities of ''on cells'' were blocked following 1.25–2.5 mg/kg morphine (Barbaro et al., 1986) while the pause in spontaneous activity of ''off cells'', triggered by noxious heat is blocked following 5 mg/kg morphine (Fields et al., 1983). These observations on ''on cells'' fit well with our results: if ''on cells'' activated by high intensities stimulation, do exert a final inhibitory effect on the reflex pathway, then the blockade of such activity by morphine will result in a facilitation of the response. The observations on ''off cells'' would also be consistent with our results, although this needs the further hypothesis of a tonic facilitation of the reflex pathway by these cells. If such a tonic facilitation is indeed interrupted by high intensity stimulation, then the blockade of this disfacilitation by morphine will result in a facilitation of the response. We do not actually know whether ''on'' or ''off'' bulbospinal neurones exhibit predominantly excitatory or inhibitory influences on the flexion reflex pathway.

In any case, the effects of systemic morphine on Rostral Ventromedial Medulla neurones could have been either direct or indirect. A direct effect is supported by the presence of some μ binding sites and numerous enkephalin terminals in the Rostral Ventromedial Medulla (Bowker et al., 1988). In particular, enkephalin-containing varicosities are numerous on the soma and proximal dendrites of ''on cells'' (Mason et al., 1992). Consistent with the hypothesis of a direct effect of systemic morphine on the Rostral Ventromedial Medulla, local administration of the μ-receptor agonist Tyr–D-Ala–Gly–MePhe–Gly-ol (DAMGO) depressed ''on cells'' and increased ''off cell'' firing, thus mimicking the effects of systemic morphine (Heinricher et al., 1994). However, identical effects were reported following microinjections of morphine within the Periacqueductal Grey (Cheng et al., 1986; Morgan et al., 1992), suggesting possible indirect effects through Periacqueductal Grey–Rostral Ventromedial Medulla–spinal pathways.

We have already discussed that the striatum and substantia nigra might well be involved in the excitatory effects of morphine on reflexes (Guirimand et al., 1995).

Interestingly in this respect, the substantia nigra sends projections to the Periacqueductal Grey (Hopkins and Niessen, 1976), a structure through which opioids have been reported to cause rigidity (Widdowson et al., 1986; Weinger et al., 1991). Note that our results are reminiscent of a report by Kuschinski et al. (1977) who recorded extensor reflex discharges following tetanic stimulation of the gastrocnemius–soleus nerve and observed a naloxone-reversible facilitatory effect of morphine (2 mg/kg, i.v.), which was blocked by spinalisation or i.p. apomorphine.

4.2. Effects of morphine during the post-conditioning period in non-transected animals

Following facilitation during the conditioning period, the reflex responses were strongly inhibited for approximately 20 min in both the intact and the sham-operated rats. This inhibition was strongly reduced by morphine in a naloxone-reversible fashion. In the Rostral Ventromedial Medulla-lesioned rats, the post-conditioning inhibition was significantly reduced and the residual inhibition was completely blocked by morphine, again in a naloxone-reversible fashion.

These data suggest that morphine decreased descending inhibitory controls triggered by temporal summation of nociceptive inputs. It appears that the morphine-induced decrease in the post-conditioning inhibition did not depend to a great extent, on the integrity of the Rostral Ventromedial Medulla. A parsimonious interpretation of these results can be found in the fact that morphine blocks Diffuse Noxious Inhibitory Controls in both animals and humans, following administration of doses low enough not to depress the spinal transmission of nociceptive signals (Le Bars et al., 1992, 1995). Interestingly, the Rostral Ventromedial Medulla is not involved in the lifting of Diffuse Noxious Inhibitory Controls by systemic morphine (Bouhassira et al., 1993b).

Taken together, these data are difficult to interpret within the framework of the hypotheses generally proposed to explain morphine analgesia in which morphine acts by increasing descending inhibitory controls from the brainstem which blocks nociceptive inflow at the spinal level (Fields and Basbaum, 1989, 1994). The arguments, which support this hypothesis, are very controversial and there are many experimental data suggesting that morphine in fact blocks descending inhibitory controls (see Refs. Duggan and North, 1984; Advokat, 1988; Le Bars et al., 1995).

4.3. Effects of morphine on the C-fibre reflex in spinal animals

In the spinal animals, the wind-up seen during the conditioning period and the post-conditioning facilitation were both reduced by morphine in a naloxone-reversible fashion. Again, the dose of morphine was too low to depress either the test responses elicited with a stimulus of 1.5 T or even the first responses during conditioning at 10 × threshold. Clearly, in this preparation, the spinal neurones were more sensitive to morphine following sensitisation by temporal summation.

During the conditioning period, the reflex responses increased from one stimulus to the next with a monotonic accelerating function, characteristic of the wind-up phenomenon. Morphine did not affect the response itself but clearly reduced the wind-up. By contrast, following intrathecal administration, morphine inhibits the steady C-fibre evoked input of dorsal horn convergent neurones but is less effective at inhibiting wind-up (Dickenson, 1991; Dickenson and Sullivan, 1986). The first explanation for such a discrepancy lies in the fact that the cited studies were in intact anaesthetised rats and we are now discussing our results in spinal non-anaesthetised animals. We have already mentioned that wind-up was not reduced by this dose of morphine in the intact anaesthetised preparation; however, such effects could have been masked by the facilitatory effects discussed above. A second explanation could perhaps be a pharmacokinetic one. It could be speculated that following intrathecal administration, morphine more easily reached the afferent fibres through the dorsal roots than the membrane of dorsal horn neurones, hence, favouring pre-synaptic rather than post-synaptic effects in the experiments conducted by Dickenson (1991) cited above. In our experiments, morphine exerted an effect on the C-fibre reflex only after the development of the ''wind-up'' process by the conditioning stimulation, and did not affect the basal test responses. One can conclude that morphine acted pre-dominantly at the post-synaptic level. Let us remember that C-fibre mediated facilitation of a flexion reflex is not due to changes in afferent terminal excitability (Cook et al., 1986). Interestingly, Silviotti et al. (1995) studied the effects of morphine on the depolarising synaptic responses of motoneurones elicited by electrical stimulation of primary sensory neurones and recorded in a hemisected spinal cord preparation in vitro. Morphine depressed the cumulative depolarisation generated by the temporal summation of synaptic responses evoked by brief 1–10 Hz trains of electrical pulses at C-fibre strength.

In spinal unanaesthetised animals, the post-conditioning inhibition was replaced by a long-lasting facilitation of the C-fibre evoked reflex, an observation consistent with several earlier studies and discussed already (Gozariu et al., 1997). The reduction in the facilitation by morphine in spinal rats is in keeping with previous results obtained with the same preparation (Blinn et al., 1980; Woolf and Wall, 1986).

In summary, the present data and our previous work (Gozariu et al., 1997, 1998) suggest that in intact rats, an inhibitory mechanism counteracts the long-lasting increase in excitability of the flexor reflex seen in spinal animals following high-intensity, repetitive stimulation of C-fibres.

Since the post-conditioning increase in excitability of the flexor reflex was very sensitive to halothane anaesthesia — as shown in our previous experiments — it is clear that the post-conditioning, supraspinally mediated, inhibitory effects were maximised in intact anaesthetised rats. On the other hand, in both the present and previous studies, the post-conditioning, spinally mediated, facilitatory effects were maximised when the animals were spinal and non-anaesthetised. Thus, we are dealing with two opposing phenomena; both are powerful, both are long-lasting but evidence for each can be obtained only in the absence of the other.

The effects of a very particular, ''turning point'' dose of morphine was intentionally chosen in order to clarify several points. Morphine increased the excitability of the spinal cord during high intensity stimulation; this effect was triggered by the strength of the nociceptive inputs and involved supraspinal structures with the Rostral Ventromedial Medulla being a key link. Wind-up was not reduced by this dose of morphine. Morphine strongly reduced the inhibition, which occurred following the conditioning period. In spinal animals, both wind-up and the post-conditioning facilitation were reduced by morphine. All effects of morphine were completely reversed by naloxone. The morphine-induced increased excitability could be related to some forms of motor stimulation elicited by opioid actions on dopaminergic transmission in the nigro-striatal system through a Periacqueductal Grey–Rostral Ventromedial Medulla–spinal pathway. The morphine-induced blockade of post-conditioning inhibitions suggests that such inhibitions were related to Diffuse Noxious Inhibitory Controls. Depressive effects of morphine were unmasked in the spinal unanaesthetised preparation. The contributions of the spinal and supraspinal effects of morphine remain to be determined in unanaesthetised freely moving animals.

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